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The primary immunodeficiency disorders

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Abstract

The primary immunodeficiency disorders are clinically heterogeneous diseases, most of which arise from inborn errors in immunologically relevant genes. A high index of suspicion is required to reach a diagnosis of primary immunodeficiency, and a timely diagnosis significantly improves patient outcomes. This contribution reviews the relationships between the underlying genetic defects and the associated immunological and clinical phenotypes seen in clinical practice. Diagnostic and therapeutic approaches to primary immunodeficiencies are also discussed.

Adrian Shields 9/6/17 15:50

Comment: We would prefer to use "the majority"

Numerically, as individual diagnostic entities, the majority of PIDs arise monogenically.

In terms of prevalence, the most common PIDs are actually polygenic (e..g CVID, sIgA def)

C.Walker 2/6/17 19:38

Deleted: the majority

Keywords

Autoimmunity; clinical immunology; genomics; infectious disease; primary immunodeficiency

Key Points

- Primary immune deficiencies present in a heterogeneous manner, and a high index of suspicion is required to diagnose primary immune deficiencies. Any patient with a suspected or proven primary immunodeficiency disorder (PID) should be referred to the care of a clinical immunologist
- Depending on the specific disease, treatments for PIDs include prophylactic antimicrobials, replacement immunoglobulin, immunosuppressive drugs, bone marrow transplantation and gene therapy
- Genomics approaches have revolutionized the diagnosis of poorly defined PID, offering patients the advantages of precise molecular diagnoses, genetic counselling and targeted immunotherapeutics

Introduction

The immune system is a complex network of cells and molecules responsible for preserving tissue homeostasis by providing defence against pathogens, performing tumour immunosurveillance and maintaining immunological tolerance. The primary immunodeficiency disorders (PIDs) are clinically heterogeneous disorders, most of which arise from genetic defects in immunologically relevant genes.

Adrian Shields 9/6/17 15:52
Comment: See above; we would prefer to use the majority

C.Walker 2/6/17 19:38
Deleted: the majority

PIDs were once thought to be exclusively associated with recurrent infections. However, as our understanding of the complexity of cellular and signalling networks has grown, it has become increasingly apparent that the clinical consequences of mutations in PID genes extend well beyond susceptibility to infection with bacteria, viruses and opportunistic organisms. *Immune dysregulation* phenotypes of PID are commonplace and include multiorgan autoimmunity, malignancy (particularly haematological) and autoinflammatory pathology such as periodic fever syndromes. These pathologies are not mutually exclusive and are often seen in combination. Furthermore, different mutations in the same gene can lead to different PID presentations, depending on whether the net effect is gain of function or loss of function at the protein level.

Adrian Shields 9/6/17 15:57
Comment: Both Dr Patel and I think that Figure 1 adds considerable value to the manuscript by highlighting the concept of immunodysregulation and immunodeficiency arising from different mutations in the same gene.

Can space be made for this to be included.

Most PIDs cause symptoms in early life. However, the heterogeneous constellation of symptoms often results in delayed diagnosis, to the patient's detriment. Adult presentations of PID tend to reflect polygenic diseases, such as common variable immunodeficiency disorder (CVID), or diseases in which environmental factors expose the underlying immunological phenotype (e.g. exposure to endemic mycobacteria can lead to the MonoMAC phenotype of GATA binding protein 2 (GATA-2) deficiency, characterized by monocytopenia and non-tuberculous mycobacterial infection).

C.Walker 8/6/17 10:58
Comment: AQ: is this the correct protein expansion? If not, please amend.

Adrian Shields 9/6/17 15:57
Comment: This expansion is correct

The advent of next-generation sequencing (NGS) has revolutionized clinical immunology by allowing detailed characterization of the genetic architecture of the immune system in healthy individuals and patients with significant immunological defects (Figure 1).[1] To date, ≥300 distinct, monogenic primary

immunodeficiencies have been described, which are classified in detail in the 2015 International Union of Immunological Societies consensus document.[2]

Below we contextualize how archetypal PIDs arise from genetic defects affecting the three stages of the immune response: (1) recognition of immunological danger, (2) immunological response to that danger, and (3) regulation of that response to restore tissue homeostasis (Figure 2). For the general physician, specific knowledge of individual PIDs is not required, but guidance on when to suspect PID and which simple investigations help in diagnosis is provided. Examples are used to highlight features specific to the perturbed stage of the immune response.

C.Walker 8/6/17 10:58
Comment: AQ: please check that the abbreviations in Fig. 2 (was 3) have been correctly defined in its footnote, and make any necessary corrections.

C.Walker 8/6/17 10:58
Comment: AQ: fig. 2, Lymphocytes column. Please provide units for lymphocyte count. Also, should TRECs be Tregs? If not, please add definition to figure footnote.

Adrian Shields 9/6/17 18:28
Comment: TREC – T cell receptor excision circle ; added to figure

Genetic defects in pathogen recognition

All immunological responses begin with the recognition of a threat to tissue homeostasis. Discrimination between immunological 'self' and 'non-self' occurs via the identification of highly conserved motifs called pathogen-associated molecular patterns (PAMPs), which are recognized by genomically encoded pattern recognition receptors (PRRs). A variety of PRRs contribute to the recognition of viral, bacterial and fungal PAMPs, the largest family being the Toll-like receptor (TLRs). Ligation of PRRs by cognate PAMPs initiates signalling cascades that shape specific immunological responses against specific pathogens.

Immunodeficiency can occur when a PRR, its subcellular localization or its downstream signalling is disrupted. For example, Mendelian susceptibility to herpes simplex virus (HSV) encephalitis can arise from genetic defects in TLR-3, a PRR that recognizes double-stranded RNA, a by-product of HSV viral replication. Individuals with TLR-3 defects suffer from recurrent, severe HSV encephalitis from an early age. The endosomal localization of TLR-3 is critical to its function, and the polytopic protein UNC-93B facilitates TLR-3 subcellular localization. Genetic mutations that prevent interaction between TLR-3 and UNC-93B produce an identical clinical phenotype to TLR-3 defects, but neither confers susceptibility to other viral infections. Such selectivity in the role of individual proteins in the defence against specific infectious disease is not unusual:

C.Walker 8/6/17 10:58
Comment: AQ: please confirm this expansion is correct, and expand if not.

Adrian Shields 9/6/17 16:10
Comment: This is correct

compound heterozygous mutations in the transcription factor IRF7, which controls type 1 interferon (IFN) responses, selectively confers susceptibility to severe influenza infection.

Other TLRs sense a variety of bacterial PAMPs including lipopolysaccharide (TLR-4), peptidoglycan (TLR-2) and flagellin (TLR-5), and signal through the Myddosome, a cytosolic, multi-protein complex that assembles following TLR ligation and initiates inflammatory gene expression. Mutations in two critical components of the Myddosome, MyD88 and IRAK-4, cause PID with identical clinical features.

MyD88 and IRAK-4 deficiencies are characterized by recurrent invasive bacterial infections including meningitis, sepsis, osteomyelitis, deep abscesses and ear, nose and throat infections. The spectrum of infection in these patients is narrow, despite the ubiquity of the Myddosome; *Streptococcus pneumoniae, Staphylococcus aureus and Pseudomonas aeruginosa* are the most commonly isolated organisms. Interestingly, patients become less susceptible with age, as adaptive immune responses develop against these organisms.

Defects in PRRs can also confer susceptibility to fungal infections. Dectin-1 senses β-1,3-linked and β-1,6-linked glucan from fungal cell walls. A severe PID involving both chronic mucocutaneous candidiasis and deep dermatophytosis can arise from mutations in the gene encoding CARD9, a downstream adapter protein employed by dectin-1 for signalling.

Genetic defects in the immunological response

Many PIDs arise from genetic mutations that prevent effective immunological responses. These defects can be classified into those affecting the innate or the adaptive immune system. Innate immunity refers to molecular and cellular mechanisms that respond rapidly and non-specifically to immunological threat. The adaptive immune system is characterized by lymphocytes that genetically rearrange their receptors (e.g. the T cell receptor (TCR) or immunoglobulin molecule) to permit the recognition of a diverse array of linear and

C.Walker 8/6/17 10:58
Comment: AQ: can this be changed to 'Myddosome', which seems to be the more usual spelling?
It appears again several times below.

Adrian Shields 9/6/17 18:21
Comment: Yes - done

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conformational epitopes and, ultimately, the establishment of long-term immunological memory. Cross-talk between the two systems is necessary for comprehensive immunological responses.

Innate immune defects

Neutrophils are the most abundant leucocytes and are rapidly recruited to sites of injury, inflammation and immunological threat to participate in early responses. The consequences of a relative or absolute absence of neutrophils (febrile neutropenia, stomatitis, sepsis) are common complications of cytotoxic chemotherapy but are mirrored in severe congenital neutropenia and cyclic neutropenia, PIDs commonly associated with mutations in the neutrophil elastase gene.

Leucocyte adhesion molecule deficiency arises if neutrophils are unable to extravasate to access sites of tissue injury, a process mediated by interactions between adhesion molecules on leucocytes and the endothelium. Mutations in integrin molecules (most commonly CD18, the β_2-integrin) impair leucocyte–endothelium interactions and neutrophil extravasation. Individuals with leucocyte adhesion molecule deficiency have a marked neutrophilia, are unable to make pus, experience delayed wound healing and have recurrent bacterial skin infections.

A key role of neutrophils is the phagocytosis and destruction of internalized pathogens, a process dependent on the generation of superoxide by the NADPH complex. Chronic granulomatous disease arises as a result of mutations within subunits of the NADPH complex. Consequently, superoxide cannot be generated, and potassium flux into the phagolysosome, which is necessary to activate destructive antimicrobial peptides, is perturbed. The clinical consequences of a defective neutrophil respiratory burst are infections and deep-seated abscesses, typically caused by catalase-positive organisms (e.g. *Staphylococcus*, *Aspergillus*, *Enterobacteriaceae* and *Candida* spp.), non-tuberculous mycobacterial infections, inflammatory granulomas and inflammatory bowel disease that mimics Crohn's

disease. Prophylactic antifungals and antibiotics are necessary, and haemopoietic stem cell transplantation (HSCT) is the treatment of choice.

Adrian Shields 9/6/17 16:25
Comment: This is spelt incorrectly. The correct spelling is haematopoetic

The innate immune system contains a vast array of effector molecules performing antimicrobial activities. For example, the complement system is a cascade of soluble plasma proteins that enhance phagocytosis by opsonization, inflammation and membrane attack. Early defects in the complement cascade (e.g. C1q deficiency) lead to childhood-onset systemic lupus erythematosus, glomerulonephritis and a predisposition to severe invasive infections. The cascade converges to assemble the membrane attack complex, comprising C5b, C6, C7, C8 and multiple C9 proteins. The complex, when assembled, allows free diffusion across the cell wall/membrane of the target. Defects in the components of the terminal complement cascade lead to recurrent meningitis with *Neisseria* spp. A diagnosis is made from the clinical history and confirmed by demonstrating abnormal haemolytic complement activity. Management involves vaccination and prophylactic antibiotics.

Cytokines are small, soluble molecules that facilitate intercellular communication within the immune system; PIDs can arise from genetic defects in their signalling pathways. For example, Mendelian susceptibility to mycobacterial disease (MSMD) confers profound susceptibility to weakly virulent, non-tuberculous mycobacteria (NTM) and arises from genetic defects in T helper type 1 cell (Th1) cytokine axes (i.e. IFN-γ, interleukin (IL)-12, IL-23). Mycobacterial infections are usually controlled by complex interactions between phagocytes and lymphocytes. Th1 cytokines, produced by Th1-polarized CD4+ T lymphocytes are instrumental in enhancing antimycobacterial activity and cell-mediated immunity. Genetic defects causing MSDM can arise at the level of cytokine receptors (e.g. IFN-γ receptors 1 and 2, common p40 subunit of the IL-12 and IL-23 receptor, IL-12 receptor chain β_1) or their downstream signalling molecules (e.g. STAT1).[3]

C.Walker 8/6/17 10:58
Comment: AQ: please confirm that this is the correct expansion and amend if not.

Adrian Shields 9/6/17 16:35
Comment: This is correct

Adaptive immune defects

Severe combined immunodeficiency (SCID) represents the most severe form of PID, and the underlying genetic defects typically prohibit early lymphocyte development.[4] The specific genetic defect determines the presence or absence of the different lymphocyte lineages (T lymphocytes, B lymphocytes, natural killer (NK) cells) and consequent immune phenotype of SCID.

Autosomal recessive mutations in the *ADA* gene allow the toxic accumulation of deoxyadenosine triphosphate and *S*-adenosylhomocysteine in developing lymphocytes, leading to apoptosis and a complete absence of T, B and NK cells. A more selective defect is seen in X-linked SCID owing to mutations in CD132, which codes for the common γ chain, which forms part of the receptors for multiple cytokines including IL-2, IL-7 and IL-15. These cytokines are critical for early T and NK cell development, but not for B cells. Defects in signalling molecules downstream of the TCR (e.g. ZAP70) and specific defects in the IL-7 pathway (IL-7RA) can lead to failure of T lymphocyte development and SCID despite the presence of B and NK cells.

Regardless of the genetic defect, the clinical presentation is similar: invasive bacterial, viral and opportunistic infections, a failure to clear live vaccines, chronic enteropathy and failure to thrive develop within the first months of life. Early diagnosis is critical to good clinical outcome. The treatment of choice is HSCT, but cell-based gene therapies, in which autologous CD34+ haemopoietic stem cells are transduced using integrative viral vectors expressing functional copies of the defective gene, have been successfully used to treat X-linked SCID and adenosine deaminase (ADA) SCID.

Adrian Shields 9/6/17 16:37
Comment: heamatopoetic

Humoral immune deficiencies are much more common and are characterized by a failure to produce effective B lymphocyte and immunoglobulin responses to pathogens. This can arise from a primary defect in B lymphocytes. For example, X-linked agammaglobulinaemia (XLA) arises from mutations in the Bruton's tyrosine kinase (*BTK*) gene. BTK is essential for B cell receptor signalling and B lymphocyte survival; loss of function leads to a complete absence of B

lymphocytes and immunoglobulins. Patients suffer from recurrent severe sinopulmonary infections and require life-long immunoglobulin replacement.

The ability of B lymphocytes to class-switch their immunoglobulin molecules from the pentameric, low-affinity IgM subclass to higher affinity IgA, IgG and IgE subclasses is essential in development of mucosal immunity and immunological memory. Affinity maturation and class switching in B lymphocytes is dependent on T-lymphocyte help, specifically the interaction between CD40 and CD40L on the surface of B and T lymphocytes, respectively. Genetic defects in CD40L or CD40 cause hyper-IgM syndrome types 1 and 2, respectively, PIDs characterized by an excess of IgM, recurrent bacterial infections and autoimmune cytopenias presenting in early childhood.

CVID is the most prevalent, clinically significant PID and is defined by marked hypogammaglobulinaemia (IgG <4.5 g/litre and reduced concentrations of either IgA or IgM), and absent responses to antigen challenge (e.g. vaccination) in patients aged >4 years. The genetic architecture of CVID is incompletely understood but most likely reflects a polygenic disease with rare variants in immunologically relevant genes, including *TACI* and *BAFFR*, conferring susceptibility but not monogenic heritability for CVID.[1] The heterogeneous clinical presentation of CVID reflects this genetic architecture.

Patients with CVID typically present in early adulthood with recurrent sinopulmonary infections. Some patients' disease does not evolve beyond this but, for reasons that remain unclear, others develop autoimmune cytopenias, increased risk of gastric and haematological malignancies, lymphocytic infiltrative diseases and granulomas. A diagnosis of CVID is made by excluding other immune deficiencies according to European Society for Immunodeficiences (ESID) registry guidelines. Treatment targets the prevention of infectious, autoimmune and structural complications and can include antibiotic prophylaxis, replacement immunoglobulin and immunosuppression.

Genetic defects in immunological regulation

Adrian Shields 9/6/17 16:38
Deleted: class-switching

Adrian Shields 9/6/17 16:42
Comment: TACI = Transmembrane activator and CAML interactor (TACI) or TNFRSF13B

Add both for completeness

C.Walker 8/6/17 10:58
Comment: AQ: this is now an alias for TNFRSF13C. Can this be added after *BAFFR*?

Adrian Shields 9/6/17 16:38
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C.Walker 8/6/17 10:58
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Adrian Shields 9/6/17 16:42
Comment: This is correct

Multiple layers of immune regulation exist to prevent autoimmunity and re-establish homeostasis following an immunological response. Interruption of these regulatory processes can lead to PIDs characterized by immune dysregulation and/or autoimmunity.

Central immunological tolerance forms the first layer of immunological regulation. During T lymphocyte development, progenitor cells are subject to complex selection processes within the thymus designed to minimize the risk of naive T lymphocytes aberrantly recognizing self-peptide in the context of the major histocompatibility complex. The transcription factor autoimmune regulator (*AIRE*) deliberately drives the expression of multiple, tissue-specific self-antigens within the thymus to negatively selective potentially autoreactive T lymphocytes.

C.Walker 8/6/17 10:58
Comment: AQ: please give MHC in full rather than abbreviated as it only appears once.

Adrian Shields 9/6/17 16:43
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Autoimmune polyendocrinopathy syndrome type 1 (APS-1), an autosomal recessive, systemic autoimmune disease, arises from mutations in the *AIRE* gene. In APS-1, negative selection of autoreactive T lymphocytes fails; these cells enter the peripheral lymphocyte repertoire and cause multiple organ-specific autoimmune diseases (e.g. type 1 diabetes mellitus, hypoparathyroidism, hypoadrenalism, vitiligo). APS-1 patients can also suffer from chronic mucocutaneous candidiasis because of a failure to establish central tolerance to IL-17, a critical cytokine in antifungal defence; autoimmunity against IL-17 develops and neutralizing autoantibodies inhibit its normal function.

C.Walker 8/6/17 10:58
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Adrian Shields 9/6/17 16:44
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Regulatory T lymphocytes (Tregs) help suppress immunological responses and maintain peripheral immunological tolerance using a variety of mechanisms. The transcription factor forkhead box P3 (*FOXP3*) is critical for the phenotypic stability of thymically derived Tregs. The rare PID immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome is caused by mutations in the *FOXP3* gene. Infants born with IPEX present in the first month of life with the triad of type 1 diabetes mellitus, severe eczema and enteropathy refractory to parenteral nutrition, and develop further autoimmune complications. Immunologically, their Treg cells are unable to control the

expansion and differentiation of T lymphocytes, which skew towards autoimmune and allergic phenotypes. HSCT is the treatment of choice.

PID can also arise if the molecular mechanisms that Tregs use to control inflammation are disrupted. CTLA-4 is an immunoregulatory cell surface receptor that competes for, blocks and transendocytoses co-stimulatory molecules from the surface of antigen-presenting cells. Signals from co-stimulatory molecules are necessary for full T lymphocyte activation, and CTLA-4 acts as a checkpoint against inappropriately excessive immunological responses. Inborn haploinsufficiency of CTLA-4 is variably penetrant but can cause multiorgan lymphocytic infiltrates, hypogammaglobulinaemia, enteropathy and autoimmune cytopenias. The subcellular trafficking of CTLA-4 from the cell surface, through the endolysosome and back to the surface is critical to its function. Mutations in LRBA, a large cytosolic protein that facilitates CTLA-4 traffic, produces an overlapping phenotype as CTLA-4 deficiency. Abatacept, a CTLA-4–Ig molecule, shows remarkable efficacy in both conditions.

During immunological responses, lymphocytes with a cognate receptor for a pathogen-derived epitope can expand a thousand-fold. Some lymphocytes survive to form immunological memory, but most are destined to die by apoptosis; contraction of clonal populations of lymphocytes is necessary to restore homeostasis. The Fas–Fas ligand axis plays a critical role in controlling clonal lymphocyte contraction. Defects in Fas, Fas ligand or caspase enzymes (caspase 8 and 10) that associate with the Fas receptor and engage apoptotic pathways can lead to autoimmune lymphoproliferative syndrome (ALPS). ALPS is characterized by widespread lymphadenopathy, cytopenias, splenomegaly, autoimmunity and an increased risk of lymphoma. Patients require immunosuppression and often HSCT.

Recognizing, diagnosing and treating primary immunodeficiency

The clinically heterogeneous presentations of PID can make them challenging diagnoses to make, and it is essential that all patients in whom PID is suspected are promptly referred to a clinical immunologist. Timely investigation and diagnosis are essential to allow early supportive interventions (prophylactic antimicrobials, avoidance of live vaccines, immunoglobulin replacement), particularly in infants, in whom fitness for curative HSCT determines clinical outcome. A growing body of evidence supports the cost-effectiveness of newborn screening for SCID using molecular analysis of lymphocytes from dried blood spot cards. However, these protocols are not yet widespread in the UK and currently only test for severe T lymphocyte defects.

A comprehensive clinical history of infections (including site, duration, responsible microorganism and response to treatment) is essential in evaluating potential PIDs. Particular attention must be paid to an individual's response to vaccinations and any features of PID or autoimmunity in their family history. Table 1 summarises the clinical features that should raise suspicion of PID and merit urgent referral to an immunology service.

Investigating immunodeficiencies is complex. Simple investigations including full blood count, renal and liver profiles, serum immunoglobulins, complement and analysis of lymphocyte subsets will reveal most common PIDs. Secondary causes such as lymphoproliferative disease, retroviral infection and iatrogenic causes should be excluded. The ESID has produced a comprehensive, patient-centered diagnostic protocol for non-immunologists investigating PID.[5] Figure 3 illustrates some of the more specialist tests used in the diagnosis of PIDs.

C.Walker 8/6/17 11:12
Comment: AQ: please complete the definitions in the caption to figure 3

Adrian Shields 9/6/17 18:25
Comment: DOne

The treatment of immunodeficiencies depends on the type, severity and clinical manifestations. The most common immunodeficiency, specific IgA deficiency, which affects 1 in 700 individuals, is usually asymptomatic, tends to be found incidentally and requires no treatment. However, most immunodeficiencies do require treatment.

With respect to infections, prophylactic antimicrobials (antibiotics, antifungals, occasionally antivirals) are used to prevent infections, and the choice of agent should be guided by previous microbiological sensitivities. Aggressive treatment of breakthrough infection is necessary, often with prolonged courses of antimicrobials. When antimicrobials alone cannot prevent infections, immunoglobulin replacement therapy can be initiated in patients with antibody deficiency or dysfunction. Immunoglobulin can be delivered intravenously or subcutaneously, depending on co-morbidities and patient preference. ADA SCID can be partially treated with replacement ADA enzyme injections, restoring the function of the defective enzyme.

HSCT remains the definitive treatment for all types of SCID and the preferred treatment option for many monogenic PIDs that present in childhood. The decision to undertake HSCT is influenced by multiple factors, not least the recipient's overall condition, their ability to tolerate myeloablative chemotherapeutic conditioning regimens and the availability of a haplotype-matched donor. Cell-based gene therapy remains in its infancy, but data from paediatric patients with SCID and chronic granulomatous disease show great promise. In the context of PID, the greatest advantage of gene therapy is that an autologous stem cell transplant can be performed with gene-modified haemopoietic stem cells, removing the requirement for a matched donor and the risk of graft rejection and graft-versus-host disease.

Adrian Shields 9/6/17 16:50
Comment: haematopoetic

An emerging field in PID is the management of complications of immune dysregulation. Immunosuppressive drugs are frequently required to treat complications of CVID such as lymphocytic infiltrative pneumonia, granulomatous disease and autoimmune cytopenias. A delicate balance must be struck between the risks and benefits of immunosuppression in patients prone to infection who also have immune dysregulation.

Special mention should be made of the role of genomics in PID.[1] The rapid reduction in cost and the speed at which genomics investigations can now be performed has revolutionized PID diagnostics and approaches to treatment.

Whole-exome and whole-genome sequencing have yielded molecular diagnoses in patients who previously had unclassified immune deficiencies, allowing informed genetic counselling for affected families and directing therapeutic management. Moreover, novel immune deficiencies defined by genomic techniques have improved our understanding of basic immunology, affording the opportunity to use rational therapeutics directly targeted at the underlying immunological defect (e.g. abatacept for CTLA-4 and LRBA mutations).

FIGURES

Figure 1 The Genomic Architecture of the Immune System

Figure 2 Archetypal primary immunodeficiencies in the context of the classical immune response

Figure 3 Laboratory investigations in primary immunodeficiencies

Adrian Shields 9/6/17 18:24
Comment: The authors think that Figure 1 makes a significant contribution to the manuscript by explaining the recent developments in immunodeficiency/immunodysregulation arising from different mutations in the same gene. This is topical and important scientifically and clinically.

Can figure 1 please be included?

Reed Elsevier 8/6/17 12:37
Comment: Chapter Editor has removed figure 1 from the article as article is over length in its current form

Reed Elsevier 8/6/17 12:38
Comment: Authors own figure.

Chapter Editor has modified the legend slightly

Reed Elsevier 8/6/17 12:24
Deleted: Figure 1 Primary immunodeficiency and immune dysregulation

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Comment: Figure 3 - the neutrophils and some of the receptors and immunoglobulin molecules are clipart Figure 4 - the neutrophil and lymphocyte drawing are clipart

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KEY REFERENCES

1. Kienzler AK, Hargreaves CE, Patel SY. The role of genomics in common variable immunodeficiency disorders. *Clin Exp Immunol* 2017; **188:** 326–32.
2. Picard C, Al-Herz W, Bousfiha A, et al. Primary immunodeficiency diseases: an update on the classification from the International Union of Immunological Societies Expert Committee for Primary Immunodeficiency 2015. *JClin Immunol* 2015; **35:** 696–726.
3. Chapman SJ, Hill AV. Human genetic susceptibility to infectious disease. *Nature Rev Genet* 2012; **13:** 175–88.
4. Rivers L, Gaspar HB. Severe combined immunodeficiency: recent developments and guidance on clinical management. *Arch Disease Child* 2015; **100**: 667–72.
5. de Vries E, European Society for Immunodeficiencies. Patient-centred screening for primary immunodeficiency, a multi-stage diagnostic protocol designed for non-immunologists: 2011 update. *Clin Exp Immunol* 2012; **167**: 108–19.

FURTHER READING

Cheng MH, Anderson MS. Monogenic autoimmunity. *Annu Rev Immunol* 2012; **30:** 393–427.

Kumar H, Kawai T, Akira S. Pathogen recognition by the innate immune system. *Int Rev Immunol* 2011; **30:** 16–34.

Kwan A, Puck JM. History and current status of newborn screening for severe combined immunodeficiency. *Semin Perinatol* 2015; **39**: 194–205.

Ochs HD, Smith CI, Puck JM. primary immunodeficiency diseases: a molecular and cellular approach, 3rd edn. Oxford: Oxford University Press, 2013.

Thrasher AJ, Williams DA. Evolving gene therapy in primary immunodeficiency. *Mol Ther* 2017; **25:** 1132–41.

TEST YOURSELF

To test your knowledge based on the article you have just read, please complete the questions below. The answers can be found at the end of the issue or online here.

Question 1

An 18-year-old man presented with a 2-day history of severe headache, fever, vomiting and meningism. At 2 years of age, he had been admitted to paediatric intensive care with septic shock, but a causative organism was not identified. His sister had died of meningitis at the age of 5 years.

On clinical examination, temperature was 38.5°C, heart rate 110 beats/minute and blood pressure 128/78 mmHg. A stiff neck was detected.

Investigations

Cerebrospinal fluid:

- Total protein 3.6 g/litre (0.15–0.45)
- Glucose 1.1 mmol/litre (3.3–4.4)
- Neutrophils 100/microlitre (0)
- Gram staining showed Gram-negative cocci

Analysis of serum showed normal concentrations of IgG, IgM and IgA.

What is the most likely diagnosis?

A. Encephalitis secondary to a TLR-3 deficiency
B. X-linked agammaglobulinaemia
C. Terminal complement deficiency
D. Chronic granulomatous disease
E. Specific IgA deficiency

Correct answer: C. The patient is likely to have a terminal complement deficiency, evidenced by recurrent meningococcal meningitis (*Neisseria meningitidis* is a Gram-negative coccus). There is no clinical evidence of encephalitis, and the cerebrospinal fluid is neutrophilic. The patient's sister also died of meningitis, making an X-linked inheritance pattern unlikely, effectively excluding the XLA and the most common genetic cause of chronic granulomatous disease. Specific IgA deficiency is effectively excluded by normal immunoglobulin concentrations.

Question 2

A 3-month-old girl presented with a 1-week history of increasing shortness of breath. She also had a 2-week history of diarrhoea. She had been previously admitted to hospital at 1 month of age with respiratory syncytial virus bronchiolitis. She was given her first set of vaccinations (UK schedule) and had two healthy siblings. On examination, she was in severe respiratory failure and was immediately intubated and ventilated.

Investigations

- *Pneumocystis jirovecii* was isolated from bronchial washings
- Immunological investigations showed complete absence of T lymphocytes, B lymphocytes and natural killer cells

What is the most likely diagnosis?

A. X-linked SCID secondary to a defect in the common γ-chain (CD132)
B. ZAP-70 SCID
C. ADA SCID
D. Common variable immunodeficiency disorder
E. Chronic granulomatous disease

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Adrian Shields 9/6/17 17:08
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Adrian Shields 9/6/17 17:18
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Adrian Shields 9/6/17 17:18
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Adrian Shields 9/6/17 17:18
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Adrian Shields 9/6/17 17:19
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Adrian Shields 9/6/17 17:19
Deleted: Urgent prophylactic antibiotics, antifungal treatment and immunoglobulin replacement therapy should be started

Correct answer: C. This girl has a severe combined immunodeficiency (SCID), evidenced by her recurrent hospital admission with both viral and fungal infections. Her diarrhoea is most likely to represent a viral enteropathy after being given live vaccination with rotavirus at 2 months of age. Defects in the common γ-chain lead to X-linked SCID, which is unlikely in a girl. The absent T, B and natural killer cells point to adenosine deaminase deficiency, which is inherited in an autosomal recessive manner, as the cause of this presentation. ZAP-70 deficiency gives a T- B+ NK+ pattern. Chronic granulomatous disease is most commonly X-linked and presents with deep-seated abscesses, typically caused by catalase-positive organisms.

Question 3

A 24-year-old man was referred to the immunology clinic with a 7-year history of chronic rhinosinusitis that had failed to improve after multiple sinus washouts. He had had two admissions to hospital in the previous 5 years with lobar pneumonia. On clinical examination, there were coarse bibasal crackles and splenomegaly.

Investigations

- Normal numbers of T and B-lymphocytes but has hypogammaglobulinaemia (low IgG and IgA but normal IgM) and does not respond to pneumococcal polysaccharide vaccination.

Sputum culture – Haemophilus influenza (resistant to amoxicillin, sensitive to co-amoxiclav, macrolides and tetracyclines)

What is the definitive long-term treatment?

A Prophylactic penicillin V
B Regular booster immunizations with pneumococcal conjugate vaccine
C Gene therapy
D Intravenous immunoglobulin replacement therapy
E Inhaled corticosteroids

Correct answer: D. The likely underlying primary immunodeficiency in this case is common variable immune deficiency disorder. The patient should be offered immunoglobulin replacement therapy with the aim of raising the IgG into the normal range, reducing the burden of infection and arresting the progress of structural lung disease. Prophylactic antibiotics may be helpful, but prophylactic penicillin V is an inappropriate choice in a patient with amoxicillin resistant Haemophilus influenzae. Booster immunizations with pneumococcal conjugate vaccine are unlikely to radically alter the disease course and as common variable immunodeficiency is polygenic, it is not amenable to current gene therapy protocols.

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Deleted: Pneumocystis should be treated and, urgent antibiotics, antifungals and immunoglobulin replacement therapy started alongside enzyme replacement before a HSCT haemopoietic stem cell transplantation is undertaken

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Adrian Shields 9/6/17 17:32
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Figure 1. The genomic architecture of the immune system. Thousands of common coding and non-coding genomic variants exist that have minor effects on an individual's immune phenotype. At the opposite end of the spectrum, there are a handful of truly monogenic PIDs with 100% penetrance (e.g. *CD132* mutations causing severe combined immunodeficiency disorder (SCID)). Between these extremes exist rare variants with significant effects on PID development (e.g. TACI C104R in CVID), variants that appear to lead to monogenic PID without 100% penetrance (e.g. *CTLA4* mutations) and PID without well-defined susceptibility genes (e.g. Specific IgA deficiency).

Figure 2. Archetypal primary immunodeficiencies in the context of the classical immune response. Classical immunological responses involve the recognition of immunological threat, a response to that threat followed by the resolution of inflammation and restoration of immunological and tissue homeostasis. Over 300 monogenic primary immune deficiencies have now been described. The clinical presentation of individual immune deficiencies is frequently attributable to how the underlying genetic defect disrupts these processes. APC, antigen-presenting cell; LoF, loss of function; MHC, major histocompatibility complex. For other abbreviations, see text.

Figure 3. Laboratory investigations in primary immunodeficiencies. Simple haematological and biochemical investigations are usually sufficient to reveal most primary immunodeficiencies. Specialist immunological investigations may be targeted at cellular or molecular components of the immune system, based on clinical suspicion. Suspected single gene defects may be confirmed by Sanger sequencing and genomics approaches are undertaken only when all other avenues of investigation have failed to yield a diagnosis. CGD, chronic granulomatous disease; CMC, chronic mucocutaneous candidiasis; DHR, dihydrorhodamine; HAE, hereditary angioedema. TREC, T cell receptor excision circle.

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Deleted: r. X-linked agammaglobulinaemia XLA is effectively excluded by the relatively late presentation and normal numbers of B -lymphocytes. Treatment should include prophylactic antibiotics, and the patient should be offered immunoglobulin replacement therapy, which will raise his IgG, but not his IgA, into the normal range as immunoglobulin products only contain only IgG. The patient has clinical evidence of complications from common variable immunodeficiency disorder, CVID including bronchiectasis and splenomegaly and will beis at increased risk of future haematological malignancies.

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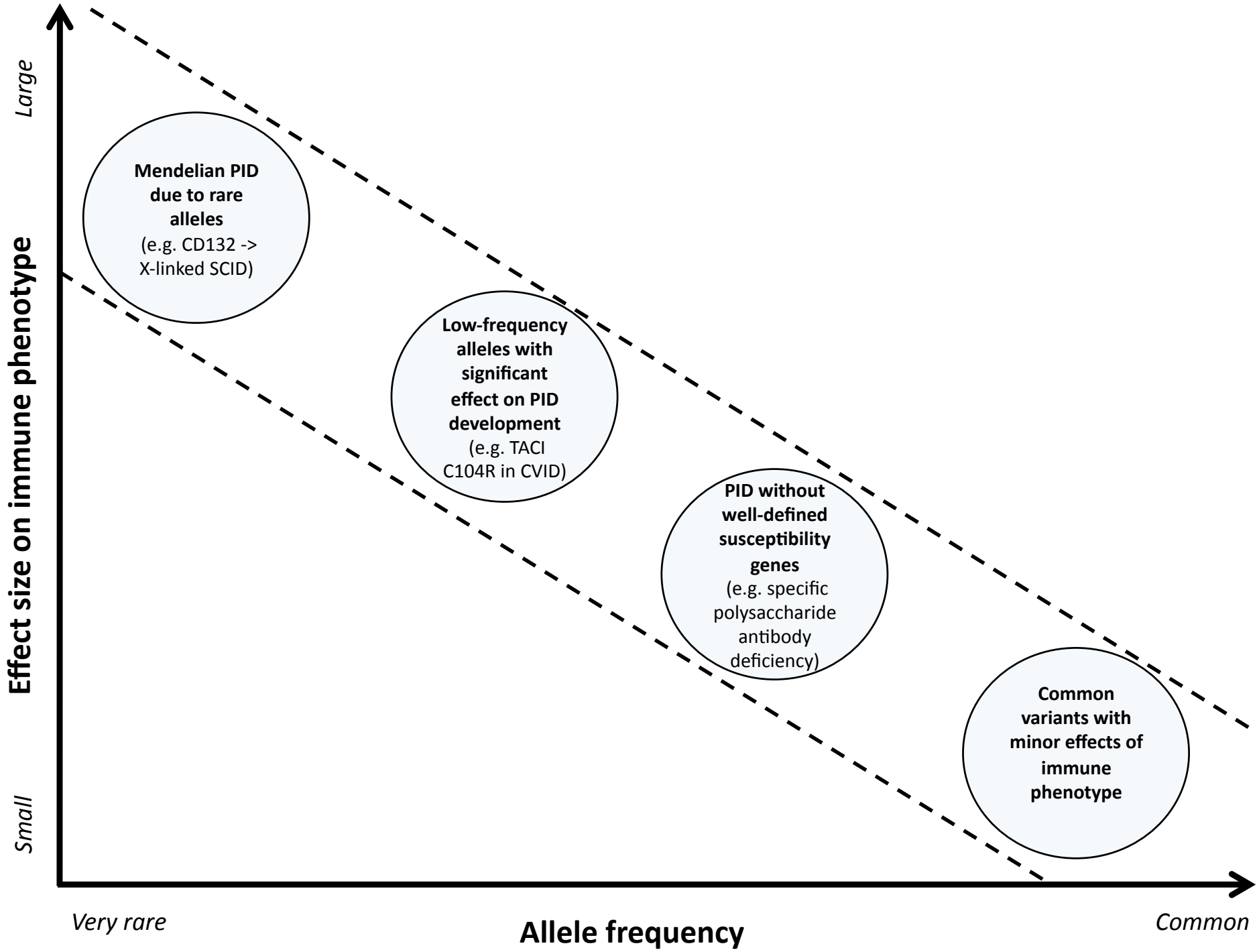
Large
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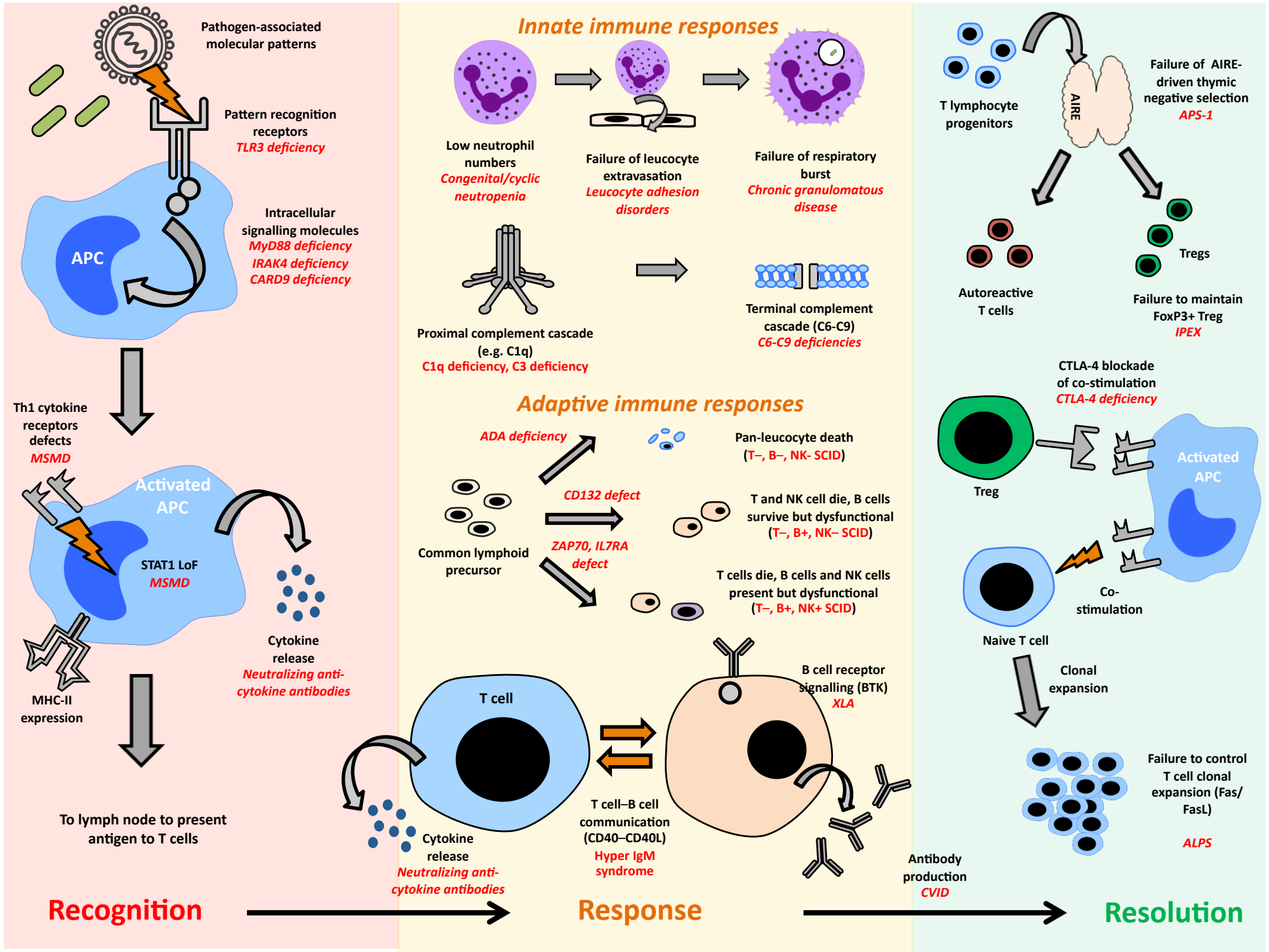
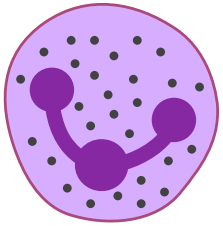
Effect size on immune phenotype
Mendelian PID due to rare alleles (e.g. CD132 -> X-linked SCID)
Low-frequency alleles with significant effect on PID development (e.g. TACI C104R in CVID)
PID without well-defined susceptibility genes (e.g. specific polysaccharide antibody deficiency)
Common variants with minor effects of immune phenotype
Very rare
Allele frequency
Common


Pathogen-associated molecular patterns
Pattern recognition receptors
TLR3 deficiency
APC
Intracellular signalling molecules
MyD88 deficiency
IRAK4 deficiency
CARD9 deficiency
Th1 cytokine receptors defects
MSMD
Activated APC
STAT1 LoF
MSMD
Cytokine release
Neutralizing anti-cytokine antibodies
MHC-II expression
To lymph node to present antigen to T cells
Recognition
Innate immune responses
Low neutrophil numbers
Congenital/cyclic neutropenia
Failure of leucocyte extravasation
Leucocyte adhesion disorders
Failure of respiratory burst
Chronic granulomatous disease
Proximal complement cascade (e.g. C1q)
C1q deficiency, C3 deficiency
Terminal complement cascade (C6-C9)
C6-C9 deficiencies
Adaptive immune responses
ADA deficiency
Pan-leucocyte death
(T–, B–, NK- SCID)
CD132 defect
T and NK cell die, B cells survive but dysfunctional
(T–, B+, NK– SCID)
Common lymphoid precursor
ZAP70, IL7RA defect
T cells die, B cells and NK cells present but dysfunctional
(T–, B+, NK+ SCID)
T cell
B cell receptor signalling (BTK)
XLA
Cytokine release
Neutralizing anti-cytokine antibodies
T cell–B cell communication (CD40–CD40L)
Hyper IgM syndrome
Antibody production
CVID
Response
T lymphocyte progenitors
AIRE
Failure of AIRE-driven thymic negative selection
APS-1
Autoreactive T cells
Tregs
Failure to maintain FoxP3+ Treg
IPEX
CTLA-4 blockade of co-stimulation
CTLA-4 deficiency
Treg
Activated APC
Co-stimulation
Naive T cell
Clonal expansion
Failure to control T cell clonal expansion (Fas/FasL)
ALPS
Resolution

Routine

Specialized immunology



Neutrophils

investigations for phagocyte disorders

Full blood count

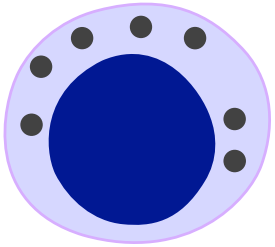
May reveal neutropenias (congenital/cyclic) or neutrophilias (LAD). Serial counts may be necessary

Respiratory burst function

Defective in CGD; assessed using DHR test (flow cytometry)

Migration, phagocytosis and killing

Defects associated with very rare PIDs; assessed using highly specialized *in vitro* tests



Lymphocytes

investigations for cellular defects

Full blood count

Lymphocyte count <2 x10^9/litre in a child <6 months is SCID until proven otherwise

Lymphocyte subsets

Flow cytometric assessment of numbers of circulating T, B and NK lymphocytes

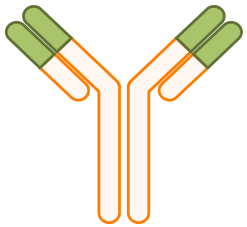
Multiple disease associations: e.g. absent B lymphocytes in XLA, absent T lymphocytes in SCID, increased CD4–CD8– T cells in ALPS

Proliferative function

In vitro tests measure lymphocyte proliferation to non-specific (mitogen) and specific stimuli (antigen) to look for specific defects

Thymic output

Molecular analysis of TRECs is helpful in defining thymic function (absent in SCID)



Immunoglobulins

investigations for humoral defects

Immunoglobulin levels

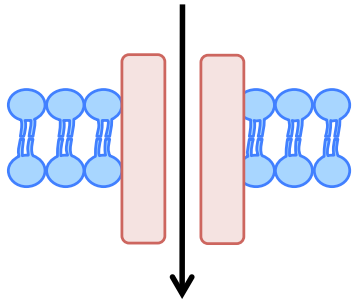
Hypogammaglobulinaemia is associated with many PIDs and confers susceptibility to infection. Hyper IgM, hyper IgE and hyper IgD syndromes may also be diagnosed based on elevations of their respective immunoglobulins

Pathogen-specific antibodies

Antibodies to specific pathogens may be assessed before and after vaccination to assess B lymphocyte function

Anticytokine antibodies

Rare PIDs are associated with the presence of antibodies against cytokines (e.g. anti-IL-17 in CMC, anti-IFN-γ in certain mycobacterial infections)



Complement

Investigations for complement defects

C3, C4 levels

Normal in terminal complement cascade defects. An isolated low C4 is associated with HAE

Haemolytic complement activity (CH50, AH50)

In vitro assays examining the classical and alternative complement cascades. Both CH50 and AH50 are absent in terminal complement defects

Quantification of individual components of complement cascade

Radial immunodiffusion assays can detect deficiencies in individual complement proteins

Genetics

Sanger sequencing and genomics investigations

A diagnosis of suspected immunodeficiency may be confirmed by Sanger sequencing a target gene to confirm a mutation. If, after exhaustive immunological investigations, a specific diagnosis can not be reached, genomic techniques including whole-exome sequencing and whole-genome sequencing may be employed.